

## THAT WHICH IS CLAIMED IS:

1. A method for analyzing the protein content of a biological sample, comprising:
  - a) separating proteins and protein fragments in the sample on the basis of chemical and/or physical properties of the proteins;
  - b) maintaining separated proteins in a separated state at discrete locations on a solid substrate or within a stream of flowing liquid;
  - c) detecting Raman spectra produced by the separated proteins at the discrete locations, wherein the spectrum from a discrete location provides information about the structure of one or more particular proteins at the discrete location.
  - c) contacting the separated proteins with capture probes under conditions suitable to form a capture probe/protein complex at one or more of the discrete locations;
  - d) contacting the complexes with a Raman-active probe construct that binds to the protein or the complex; and
  - e) detecting Raman spectra produced by the probe construct/protein complexes at the discrete locations, wherein the spectrum from a discrete location provides information about the structure of one or more particular proteins at the discrete location.
2. The method of claim 1, further comprising correlating the information with information regarding source of the sample.
3. The method of claim 2, wherein the capture probe is a primary antibody that binds specifically to the protein in the complex.
4. The method of claim 1, wherein the a Raman-active probe construct comprises a secondary antibody as probe and one or more Raman tags.

5. The method of claim 4, wherein the Raman-active probe construct is a COIN with a unique SERS signature and the Raman spectrum detected is a SERS spectrum.
6. The method of claim 1, wherein the proteins are solubilized in an aqueous solution or hydrophilic solvent prior to the separation.
7. The method of claim 1, further comprising denaturing the proteins in the sample prior to the separation.
8. The method of claim 7, wherein the denaturing agent is selected from a reducing agent, a surfactant, a chaotropic salt, and a combination thereof is used to denature the proteins.
9. The method of claim 8, wherein denatured proteins are dried on the substrate prior to the detection of signals.
10. The method of claim 1, wherein the substrate is coated with one or more organic or inorganic materials prior to immobilization of the proteins thereon.
11. The method of claim 10, wherein the separated proteins are deposited at the discrete locations on the solid substrate by a procedure selected from contact writing, contact spotting, liquid spraying, and dry particle spraying.
12. The method of claim 1, wherein the separated proteins are deposited without denaturing using wet electrospray deposition.
13. The method of claim 1, wherein the substrate is aluminum.
14. The method of claim 1, wherein the substrate is comprised of a plurality of the discrete locations on a flat plate.
15. The method of claim 1 or 14, wherein the detecting is automated to accomplish high throughput scanning at sequential discrete locations.

16. The method of claim 1, wherein the discrete locations on the substrate comprise a material selected from gold, silver, copper, and aluminum metals, glass, silicon, and ceramic materials.
17. The method of claim 1, further comprising contacting the proteins at the discrete locations with silver nanoparticles, in individual or aggregate forms.
18. The method of claim 17, further comprising contacting the nanoparticles with at least one chemical enhancer salt.
19. The method of claim 18, wherein the chemical enhancer salt is LiCl.
20. The method of claim 17 or 18, wherein the Raman spectra are SERS spectra.
21. The method of claim 1 or 17, further comprising collecting the Raman spectra or SERS spectra from the discrete locations to compile a protein profile of the sample.
22. The method of claim 21, wherein the collection is automated to accomplish high-throughput SERS spectra screening of the discrete locations.
23. The method of claim 1, wherein the relation between SERS spectra and sample locations are recorded and correlated.
24. The method of claim 1 or 22, wherein the spectrum contains information regarding a protein characteristic selected from a chemical bond, residue composition, residue structure, relative positions of residues, identity of the protein, and combinations thereof.
25. The method of claim 1, wherein the separated proteins are maintained in a separated state by sequentially introducing the separated proteins or fragments into the flowing stream to form the discrete locations.
26. The method of claim 25, further comprising mixing the stream of separated proteins with a stream of metal colloids under conditions suitable for formation of SERS-active nanoparticles and the detection is SERS detection.

27. The method of claim 1, further comprising analyzing the separated proteins by mass spectroscopy to identify one or more functional groups contained within a separated protein or fragment thereof.
28. The method of claim 27, further comprising compiling data obtained from the Raman spectra or SERS spectra with data obtained from the mass spectroscopy.
29. The method of claim 1 or 28, wherein the sample is a patient sample.
30. The method of claim 29, wherein the patient sample is a body fluid selected from urine, blood, plasma, serum, saliva, semen, stool, sputum, cerebral spinal fluid, tears, and mucus.
31. The method of claim 1 further comprising creating a protein profile of the sample based on data obtained from the Raman spectra and/or the SERS spectra.
32. The method of claim 31, further comprising repeating the method using a variety of different patient samples to create a protein library containing a plurality of different protein profiles.
33. The method of claim 32 further comprising comparing the protein profile of the sample with one or more protein profiles of the library to detect a difference, wherein the difference is indicative of a disease in the patient.
34. A kit for analyzing the protein composition of a complex mixture of proteins, comprising:
  - a) a substrate having a plurality of discrete locations that are coated with positively charged or negatively charged compounds, or with neutral or hydrophobic polymers for immobilization of proteins and protein fragments at the discrete locations; and
  - b) a container holding nanoparticles of silver, gold, copper or aluminum.
35. The kit of claim 34, further comprising a protein denaturing agent.

36. A system for analyzing the protein composition of a complex mixture of proteins comprising:

- a) a substrate having a plurality of discrete locations having a coating selected from a metal layer, a positively charged or negatively charged compound, and neutral or hydrophobic polymers for immobilization of proteins and protein fragments;
- b) a sample containing at least one protein-containing compound;
- c) a Raman spectrometer; and
- d) a computer comprising an algorithm for analysis of the sample.

37. The system of claim 36, wherein the Raman spectrometer is a scanner of SERS signals received consecutively from a plurality of the discrete locations.